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Optical Recording of Electrical Activity

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Foreword

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More than three decades have passed since the introduction of potentiometric dyes for monitoring electrical activity in cells and tissues. At its inception, optical measurement of membrane potential promised advantages under circumstances where electrodes were difficult to use for reasons of size, complexity, or membrane topology. While many of these benefits have been realized, what is most remarkable today is the number of applications that were not, or could not have been, anticipated. Many of these are to be found in the papers contained in this special issue of the Journal of Membrane Biology. It is particularly appropriate that they should be reported here, as it was in this journal that the earliest progress in the search for new and more voltage-sensitive dyes was documented.

The fastest potentiometric probes respond to step changes in membrane voltage in less than 1–2 μs, and the temporal resolution of optical recording was always known to be limited, primarily, by the bandwidth of the measurement, typically constrained by signal-to-noise considerations. The spatial resolution, however, was assumed without justification, to reflect microscope optics, with optical measurement of membrane potential possible at the cellular and just sub-cellular level. Triumphantly, the contribution by Blunck et al. demonstrates how voltage-sensitive dyes can, instead, be used to probe the local electric field strength even within a single anisotropic membrane protein, an ion channel. Another refinement of the optical recording approach that was not anticipated in 1973, or for many years thereafter, is the potential for genetic encoding of one or more components of a molecular probe system, with the implied capability of targeting an indicator to a selected subset of cells, or to specific regions of cells. These authors also discuss one particularly elegant solution to this quest using, as a voltage-sensitive Förster Resonance Energy Transfer (FRET) acceptor, a highly mobile but non-fluorescent synthetic anion, dipicrylamine. The genetically targeted fluorescence donor in this hybrid scheme is a modified eGFP that can be localized exclusively to the inner leaflet of the plasma membrane.

Thirty years ago, the first sensitive potentiometric dyes were fluorescent molecules such as Merocyanine 540 and its numerous analogues. Pure absorbance dyes quickly followed, and linear and quadratic mechanisms were proposed to account for their electric field sensitivity. However, the possibility of exploiting intrinsically non-linear optical phenomena like second harmonic generation (SHG) hadn't even been imagined. The paper by Millard et al. examines what can be termed "resonance-enhanced" SHG from membrane-bound styryl dyes, and shows that potentiometry, which exploits the center-asymmetry of dyes bound to only a single membrane leaflet, can be more sensitive to membrane voltage than that based upon fluorescence.

Among the earliest suggested applications of voltage-sensitive dyes was their potential use in probing membrane voltage at otherwise inaccessible regions of cells, perhaps best exemplified by the transverse tubule system of skeletal muscle. This possibility was realized quite early on, but, as the paper by DiFranco et al. makes abundantly clear, fluorescent potentiometric indicators, combined with voltage-clamp technique, now provide new and unexpected insight into the kinetics of activation of the transverse tubular system of mammalian muscle.

While the primary focus of this special issue is on optical recording of membrane potential, the contribution by Kosterin et al. uses a novel intrinsic fluorescence signal to explore the coupling between the

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action potentials, recorded optically in mammalian neurosecretory terminals, and the metabolic changes that follow in the terminal mitochondria. These authors demonstrate that it is the rise in intraterminal Ca^{2+}, in conjunction with the increase in ADP mediated by the turnover of the Na^{+}/K^{+} ATPase, that together act to apprise the mitochondria of the level of neurosecretory activity.

In more than three decades, several thousand plausibly potentiometric probes have been synthesized and assayed. The vast majority of these dyes absorb and emit in the visible region of the spectrum and many have found application in a great variety of studies of cell and tissue physiology. There has always been the hope, however, that the spectral range of voltage-sensitive dyes could be extended far into the red and beyond. Not only would this permit molecular probes to be used in tissues like the retina that are sensitive to photons in the visible, but longer wavelength excitation and emission would enable enhanced light penetration in three-dimensional structures like the heart, where the spatiotemporal patterns of activity are neither simple nor always superficial. In this issue Salama et al. report new, very long wavelength voltage-sensitive dyes, and illustrate their use in cardiac muscle and in a neurosecretory organ.

From the earliest reports of optical probes of membrane potential, ambitious ideas for the application of these molecular indicators to the study of the central nervous system were never far from consciousness. The tantalizing metaphor of Sherrington's "enchanted loom where millions of flashing shuttles weave a dissolving pattern, always a meaningful pattern though never an abiding one; a shifting harmony of subpatterns" drove scientists to find new ways of exploiting voltage-sensitive dyes to learn more about the brain, that "great ravelled knot". The three final contributions to this special issue illustrate some of the new directions that are now becoming practicable. Milojkovic et al. have been able to identify a completely novel and unexpected form of electrical signal in the cerebral cortex; a signal that is qualitatively and quantitatively different from back-propagating action potentials and dendritic plateau potentials. On a larger cortical scale, Civilico and Contreras have explored the relationship between the responses of somatosensory cortex (whisker barrels) to physiological inputs and to electrical stimulation. Since direct electrical stimulation may, and most likely, does, initiate combinations of synaptic events that would never occur in response to physiological stimuli, the use of optical recording to monitor the amount of cortical area activated, and the temporal relations among activated regions is absolutely critical. Finally, Momose-Sato and Sato demonstrate the use of optical recording to identify novel functional synaptic connections made by the vagus nerve in an hitherto neglected region of the brainstem. These observations, barely conceivable without functional optical mapping using potentiometric probes, suggest that sensory information processing mediated by the vagus nerve is more complex than previously understood.

From intramolecular profiling of the dynamic electric field, to cortical and sub-cortical mapping of brain activity, this special issue provides a tantalizing glimpse at the still largely unrealized potential of optical recording. The guest editor hopes that the reader will find these developments as interesting as he finds them, and that the dying words of Johann Wolfgang von Goethe will continue to inspire: "Mehr Licht."